

CLAIMS

1. A method of determining the ability of a test compound to affect the activity of ABCA1 protein, said method comprising the steps of:

- 5 a) introducing labeled substrate into ABCA1-expressing cells;
- b) adding a test composition comprising said test compound to a first portion of said cells, and adding a control composition to a second portion of said cells, wherein said control composition is essentially identical to said test composition except that said control composition does not include said test compound; and
- c) comparing the level of efflux of substrate from said first portion of said cells to the level of efflux of substrate from said second portion of said cells, wherein a change in the level of efflux indicates that said test compound affects the activity of the ABCA1 protein.

15 2. The method of claim 1, wherein said substrate is cholesterol or a phospholipid.

3. The method of claim 1, wherein said cells are mouse macrophage J774 cells, monocytes, macrophages, hepatocytes, endothelial cells, fibroblasts, or enterocytes.

20 4. The method of claim 1 wherein said substrate is labeled with tritium, carbon-14, deuterium, a fluorescent tag, or a luminescent tag.

25 5. The method of claim 1 wherein a positive control composition is added to a third portion of said cells, said positive control composition being essentially identical to said control composition except that said positive control composition comprises an ABCA1 agonist, and wherein the level of efflux of substrate from said third portion of said cells is compared to the levels of efflux of substrate from said first and said second portions of said cells.

Sub
An
Treated
Untreated

6. The method of claim 5 wherein said ABCA1 agonist is cAMP, cpt-cAMP, vanadate, protein kinase A, okadaic acid, prostaglandin E1, or PDE inhibitors.

7. The method of claim 1 wherein a negative control composition is added
5 to a third portion of said cells, said negative control composition being essentially identical to said control composition except that said negative control composition comprises an ABCA1 antagonist, and wherein the level of efflux of substrate from said third portion of said cells is compared to the levels of efflux of substrate from said first and said second portions of said cells.

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8. The method of claim 7 wherein said ABCA1 antagonist is glibenclamide, sulfobromophthalein, flufenamic acid, diphenylamine-2-carboxylic acid, DIDS, bumetanide, or furosemide.

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9. The method of claim 1 wherein the level of efflux of substrate from each portion of said cells is measured prior to said comparing step c).

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10. The method of claim 9 wherein the level of efflux of substrate from each portion of said cells is measured by separately combining said first and said second portions of said cells with an efflux media, said efflux media comprising an ABCA1 acceptor, and measuring the amount of substrate that associates with said ABCA1 acceptor in each of said portions of said cells.

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11. The method of claim 10 wherein said ABCA1 acceptor is apo-AI, apo-AIV, apo-Cl, apo-CII, apo-CIII, apo-E, apo-E2, apo-E3, apo-E4, or a synthetic amphipathic peptide representing the alpha-helical domain of an apoprotein.

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12. The method of claim 11 wherein said ABCA1 acceptor is apo-AI.

13. The method of claim 10 wherein the amount of substrate that associates with an acceptor for ABCA1 is measured by determining the amount of labeled substrate that appears in the medium of the cells.

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Sub A2

Sub A3

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14. The method of claim 1 wherein a positive control composition is added to a third portion of said cells, said positive control composition being essentially identical to said control composition except that said positive control composition comprises an ABCA1 agonist, a negative control composition is added to a fourth portion of said cells, said negative control composition being essentially identical to said control composition except that said negative control composition comprises an ABCA1 antagonist, and wherein the levels of efflux of substrate from said third and said fourth portions of said cells are compared to the levels of efflux of substrate from said first and said second portions of said cells.

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15. A method of determining the ability of a test compound to affect the activity of ABCA1 protein, said method comprising the steps of:

- introducing labeled anions into ABCA1-expressing cells;
- adding a test composition comprising said test compound to a first portion of said cells, and adding a control composition to a second portion of said cells, wherein said control composition is essentially identical to said test composition except that said control composition does not include said test compound; and
- comparing the level of efflux of anions from said first portion of said cells to the level of efflux of anions from said second portion of said cells, wherein a change in the level of efflux indicates that said test compound affects the activity of the ABCA1 protein.

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18. A method of determining the ability of a test compound to affect the transcription of ABCA1 mRNA, said method comprising the steps of:

- adding a test composition comprising said test compound to a first portion of ABCA1-expressing cells, and adding a control composition to a second portion of said cells, wherein said control composition is essentially identical to said test composition except that said control composition does not include said test compound; and
- comparing the amount of ABCA1 mRNA from said first portion of said cells to the amount of ABCA1 mRNA from said second portion of said cells,

wherein a change in the amount of ABCA1 mRNA indicates that said test compound affects the transcription of ABCA1 mRNA.

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5 A method of determining the ability of a test compound to affect the expression of ABCA1 protein, said method comprising the steps of:

10 a) adding a test composition comprising said test compound to a first portion of ABCA1-expressing cells, and adding a control composition to a second portion of said cells, wherein said control composition is essentially identical to said test composition except that said control composition does not include said test compound; and

c) comparing the amount of ABCA1 protein from said first portion of said cells to the amount of ABCA1 protein from said second portion of said cells, wherein a change in the amount of ABCA1 protein indicates that said test compound affects the expression of the ABCA1 protein.